

Poietics™ Human Visceral Preadipocytes HPrAD - Instructions for Use

Introduction

Primary Human Visceral Preadipocytes are isolated from visceral (kidney and bladder) adipose tissue by enzymatic digestion and selective culturing techniques. Unpassaged Primary Human Visceral Preadipocytes are cryopreserved in a solution containing EGM™-2 MV, FBS and DMSO.

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. BulletKit™ Instructions: Upon arrival, store basal medium at 4-8°C and SingleQuots™ at 20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 4°C and added to basal medium within 72 hours of receipt. After SingleQuots™ are added to basal medium, use within one month. Do not re-freeze.

Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

4. Dulbecco's Phosphate Buffered Saline can be stored at ambient temperature. The Trypsin/EDTA Solution is sterile filtered and then stored at -20°C until shipment. Trypsin/EDTA may thaw during transport. It may be refrozen once. Trypsin/EDTA Solution has a limited shelf life or activation at 4°C. If, upon arrival,

Trypsin/EDTA is thawed, immediately aliquot and refreeze at -20°C.

NOTE: To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at -20°C.

Using media or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

Preparation of Media

1. Use pre-warmed (37°C), supplemented medium for culturing preadipocytes
2. Decontaminate the external surfaces of a 500 ml bottle of Preadipocyte Basal Medium-2 with 70% v/v ethanol or isopropanol.
3. Make up Preadipocyte Growth Medium-2 by adding the entire contents of the FBS, L-glutamine, GA-1000 SingleQuots™ to the bottle of Preadipocyte Basal Medium-2 (The final concentrations of the supplements will be 10%, 2 mM, 30 µg/ml and 15 ng/ml respectively). Reserve 100 ml of Preadipocyte Growth Medium-2 for subsequent preparation of differentiation medium.

Thawing of Cells / Initiation of Culture Process

NOTE: The thawing procedure described below has been developed to provide optimal recovery and cell viability. Failure to follow this protocol will result in lower yields of viable progenitor cells.

1. Remove the cryovial of cells from liquid nitrogen storage and thaw rapidly in a 37°C water bath for no more than 2 minutes. Decontaminate the external surfaces of the cryovial of cells with 70% v/v ethanol or isopropanol.

2. Using a micropipette, gently add the thawed cell suspension to 50 ml of pre-warmed Preadipocyte Growth Medium-2.
3. Rinse the cryovial with medium and add the rinse to the cell suspension.
4. Centrifuge at 300 x g for 10 minutes at room temperature.
5. When washing the cells, do not attempt to remove too much of the wash. Leave a minimum of 1 ml of wash at the bottom of the tube. If the final cell count is low, some of the pellets may have been removed with the wash.
6. Add 2 or 3 ml of Preadipocyte Growth Medium-2 to the 1 ml of wash and resuspend the pellet of cells. Dilute 20 μ l of the cell suspension in 20 μ l of 0.4% Trypan Blue; do a cell count and determine % viability. Recovery should be approximately 90%.

If the cells are to be expanded prior to use for assays:

7. Plate the preadipocytes at approximately 9,000 cells/cm² in 0.2 ml medium/cm² growth area in tissue culture flasks and gently rock to disperse the cell suspension over the growth surface.
8. Human primary preadipocytes will appear round when first plated. Within four hours, greater than 90% of the cells should be attached and will begin to flatten and elongate. Within 24-36 hours, the cells will be adherent, elongated and spindle shaped. As preadipocytes begin to divide, they will round up. During mitosis, the cells stay loosely attached and once division is completed, will again flatten and elongate.
9. Preadipocyte cultures should be fed every 3-4 days after plating.
10. The preadipocyte culture will have a doubling time of approximately 36 to 48 hours. Preadipocyte cultures should be cultured to 70% confluency – do not allow the cells to become confluent as they will undergo growth arrest, a precursor to differentiation. At this point, the cells can be harvested and either used in assays or subcultured.

Subculturing

1. Aseptically remove and discard all of the spent media from the flasks.
2. Wash the attached cell layer with Dulbecco's Phosphate Buffered Saline (# 17-512F) or an

equivalent calcium and magnesium free balanced salt solution. Add the wash solution to the side of the flask opposite the attached cell layer. Rinse by rocking the flask back and forth several times. Aseptically remove and discard the wash solution.

3. Add a sufficient volume of Clonetics™ Trypsin-EDTA solution, (CC-3232), to cover the cell layer (approx. 25 μ l/cm²). Gently rock the flask(s) to ensure that the cells are covered by the trypsin solution.
4. Incubate at room temperature for 3 minutes, then observe under a microscope. If the cells are less than 90% detached, continue incubating and observe every minute. Tapping the flask or plate will expedite cell detachment. Do not incubate the cells longer than 15 minutes.
5. Once >90% of the cells are rounded and detached, stand the flasks on end and add temperature equilibrated Preadipocyte Growth Medium-2 to each flask (approximately 0.2 ml/cm²). Disperse the solution by pipetting over the cell layer surface several times.
6. To remove the trypsin, centrifuge cells at approximately 300 x g for 10 minutes at room temperature.
7. Resuspend the cell pellet(s) in a minimal volume of temperature equilibrated Growth medium and remove a sample for counting.
8. Count the cells with a hemacytometer or cell counter and calculate the total number of cells. Make a note of cell yield for later use.
9. Primary human preadipocytes should not be passaged more than once.

If the cells are to be assayed immediately:

10. Resuspend the preadipocytes at 100,000 cells/ml in Preadipocyte Growth Medium-2 and plate the preadipocytes at 10,000 cells/well of a 96-well cell culture plate in 0.1 ml of Preadipocyte Growth Medium-2.
11. Incubate at 37°C, 5% CO₂ and >90% humidity until the cells are confluent.

Maintenance / Differentiation

To Prepare Adipocyte Differentiation Medium

1. Add the entire contents of the SingleQuots™ of insulin, dexamethasone, indomethacin and isobutyl-methylxanthine to 100 ml of

Preadipocyte Growth Medium-2 prewarmed to 37°C.

The PGM™-2 contains insulin and that elimination of the insulin SingleQuots™ in the Differentiation Medium will not make the resulting Differentiation Medium insulin-free.

NOTE: The 100 ml of Differentiation Medium will be “2X” – the concentrations of the differentiation agents will be diluted 2-fold when added to the pre-plated cells.

Differentiation of Cells

1. Induce the preadipocytes to begin differentiating into adipocytes with the addition of 0.1 ml of Adipocyte Differentiation Medium to each well. Note that the differentiation agents in the Differentiation Medium will be diluted 2-fold.
2. If the cells are to be treated with a series of test samples, set up several 24-well dilution plates with the appropriate volume of Preadipocyte Differentiation Medium/well and make the required serial dilutions of the test samples. Add 0.1 ml of each different concentration of test samples to wells of the pre-seeded confluent preadipocytes. Each assay should be done in triplicate.
3. We suggest that “control” wells be set up which contain 1) 100 µl of Preadipocyte Growth Medium-2 instead of Differentiation Medium 2) no added test sample and 3) “solvent only” if the test samples were dissolved in solvents such as DMSO, ethanol, etc.
4. No further additions or medium changes are required. Differentiated adipocytes are delicate and care should be used to avoid disrupting the numerous lipid vacuoles in the cells.
5. The extent of adipocyte differentiation may be noted by microscopic observation of lipid vacuoles in the induced cells. The intracellular lipid vacuoles will begin to appear 4 to 5 days after induction and will continue to increase in number and size for 7 to 10 days. Non-induced cells will have few, if any, lipid vacuoles.
6. To document adipocyte differentiation, cultures may be carefully rinsed with PBS, fixed with 10% buffered formalin and stained with Oil Red O. Poietics™ AdipoRed™ Reagent (PT-7009) is a recommended and convenient reagent for the high-throughput assay of *in vitro* preadipocyte differentiation. Alternatively, adipocyte differentiation can be measured by immunoassays or mRNA amplification assays to quantify the expression of proteins such as

leptin, AP2 or PPAR γ that have been used as “markers” of the differentiated adipocyte.

Quality Control (QC)

For detailed information concerning QC testing, please refer to the Certificate of Analysis.

Ordering Information

Cryopreserved Cells

Cat. No.	Product	Description
PT-5005	HPrAD-vis, Visceral Preadipocytes	≥ 1,000,000 cells
PT-5023	HPrAD-vis, Visceral Preadipocytes (Diabetes Type I)	≥ 1,000,000 cells
PT-5024	HPrAD-vis, Visceral Preadipocytes (Diabetes Type II)	≥ 1,000,000 cells

Related Products

Preadipocyte Growth Media (Must be purchased separately)

Cat. No.	Product	Description
PT-8002	PGM™-2 BulletKit™	Kit which contains a 500 ml bottle of PBM-2, (PT-8202) and PGM™-2 SingleQuots™ (PT-9502).
PT-8202	PBM-2	Preadipocyte Basal Medium-2 (no growth factors) (500 ml)
PT-9502	PGM™-2 SingleQuots™	Supplements and growth factors (FBS, L-glutamine, GA-1000, Insulin, Dexamethasone, Indomethacin, 3- Isobutyl-1-methylxanthine)
PT-7009	AdipoRed™ Test Kit	5 X 4.0 ml

Subculturing Reagents (sold separately)

Cat. No.	Product	Description
17-512F	Dulbecco's Phosphate Buffered Saline	500 ml
CC-3232	Trypsin/EDTA Solution	100 ml

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza guarantees the performance of its cells only if Poietics™ Media and Reagents are used exclusively, and the recommended protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete Cell System. Cryopreserved PrAD-vis cells are assured to be viable and functional when thawed and maintained properly.

Safety Statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing cannot offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 5th Edition. If you require further information, please contact your site Safety Officer or Scientific Support.

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